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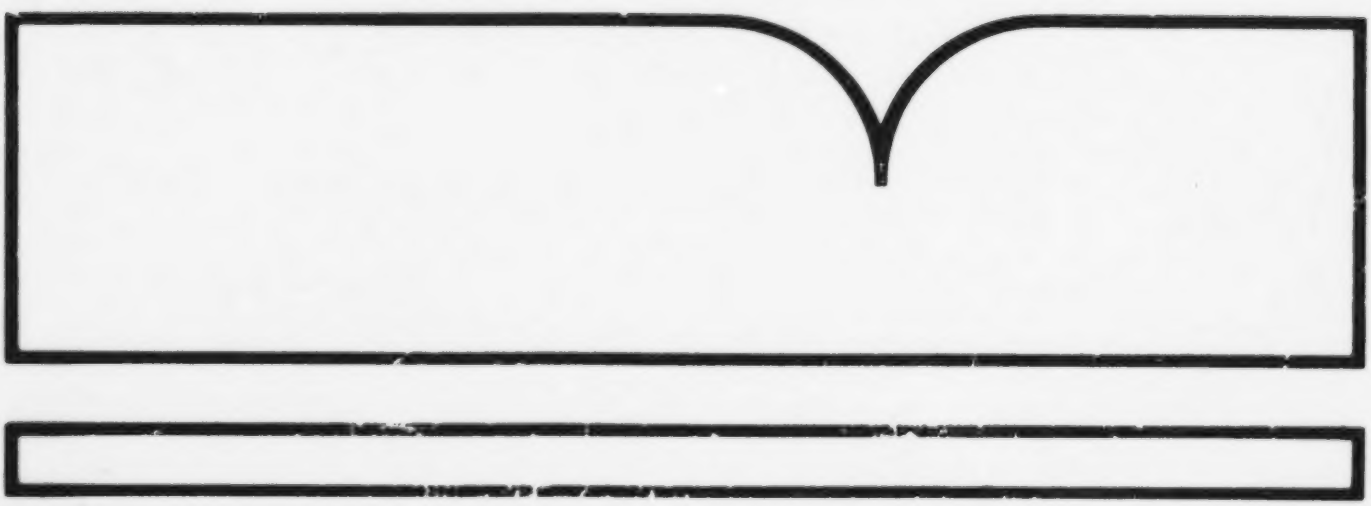

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CONTROL OF WATERBORNE *CAMPYLOBACTER JEJUNI* BY CHLORINE DISINFECTION

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16. ABSTRACT

Results of preliminary studies on the comparative sensitivity of Campylobacter jejuni and Escherichia coli to inactivation by chlorine are presented. The results indicate that C. jejuni is equally or more sensitive to inactivation by free chlorine as the E. coli test strain. Chlorination procedures employing free chlorine that are effective for inactivation of E. coli should also be effective for inactivating the pathogen C. jejuni.

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CONTROL OF WATERBORNE *CAMPYLOBACTER JEJUNI* BY CHLORINE DISINFECTION

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Campylobacter jejuni and closely-related organisms are the most common bacterial causes for acute enteritis in the United States (1). Transmission of *Campylobacter jejuni* from contaminated potable water supplies and foods is well documented (2). In recent years, several outbreaks have been reported in which municipal water supplies have been implicated as the source for 200-3,000 symptomatic infections (2). Most cases of waterborne *Campylobacter* infection have occurred as a result of ingestion of untreated or improperly-treated water. Because source waters for municipal drinking systems may be contaminated with *C. jejuni* (3), it is important to document the efficacy of the usually employed disinfecting agents for killing these organisms.

Chlorine (hypochlorous acid [HOCl]), generally accepted as a universal water disinfectant is an effective bacteriocidal agent against a variety of enteric pathogens. In preliminary studies, under a limited range of experimental conditions, we showed that 3 strains of *C. jejuni* were susceptible to the bactericidal effects of chlorine (4). This report describes the results of a study to determine the effect of chlorine treatment on *C. jejuni* under conditions simulating those that would be encountered during routine treatment of source waters.

METHODS

Bacteria. The *C. jejuni* organisms used were the type strains PEN 1, PEN 2, and PEN 3 of the PENNER serotyping system representing three of the most common *C. jejuni* isolates of humans (5). *Escherichia coli* ATCC 11229 was used as a control. The *C. jejuni* and *E. coli* strains were grown on blood agar according to previously described methods (4). Overnight cultures were harvested by gently scraping from plates in chlorine demand-free water, washed by centrifugation and resuspension three times and enumerated as described previously (4).

Reagents. Chlorine-demand-free water was prepared as previously described

(6). Phosphate buffers (pH 6.0 - pH 8.0) were made using the chlorine-demand-free water. Chlorine stock solutions were made using sodium hypochlorite in the desired phosphate buffers. Reaction mixtures were quenched in 0.1% sodium thiosulfate.

Assay. Log dilutions of the C. jejuni and E. coli cells were made to reach a final concentration of 10^3 - 10^5 colony-forming units (cfu)/ml. The sodium hypochlorite solutions in buffer were standardized using amperometric titration (7) to reach the desired concentrations. All solutions were tested for free available chlorine and chlorine-demand just prior to use in the assay. Incubation temperature of the reaction mixtures was at 5oC and 25oC. Initial studies were done with contact times of 0, 5, 15, and 30 minutes but later studies examined the interaction during the first 5 minutes. At the end of each period of contact, 1.0 ml of each reaction mixture was added to 1.0 ml of the sodium thiosulfate solution, and then serial ten-fold dilutions taken, and 0.1 ml aliquots from each were inoculated in duplicate by spread plating onto blood agar plates, incubated, and the colonies enumerated. Determination of chlorine binding during the contact period was done using the DPD method (7).

RESULTS

Interaction of test strains with thiosulfate. The first studies done were to determine whether the use of 0.1% sodium thiosulfate to neutralize free residual chlorine would have any inhibitory effect on the test strains. Results are shown in Figure 1, which demonstrated that neither the PEN 1 C. jejuni nor the E. coli strain were significantly inhibited. During the experimental disinfection procedures, the maximum period of contact with the thiosulfate was under 30 minutes, well within the period of no inhibition.

Chlorine-demand. Before proceeding with the chlorine disinfection studies we next examined the question whether after the introduction of either E. coli or C. jejuni into the chlorine-demand-free water or buffers in our assay system chlorine-demand would be seen. The results of these studies are shown in Tables 1 and 2. In general, there was a dose-response relationship in which the heavily seeded water showed demand and somewhat less was noted when smaller seed dilutions were used (data not shown). Within the range tested, there was no consistent effect of pH on the binding reaction. Studies done at 25oC showed similar binding.

Chlorine disinfection. Figures 2 and 3 represent the format and results of the disinfection studies. For each combination of variables, a single study was done. As shown in Figures 2 and 3 after contact of the bacterial cells and the free chlorine-containing solution, binding occurred rapidly with nearly all of the loss of free chlorine residual occurring within the first minute. Enumeration of the test strains in buffer without chlorine showed no killing during the course of the study. At pH 8, there was >4 log $_{10}$ killing of E. coli and C. jejuni within 5 minutes, but killing occurred more rapidly for C. jejuni than for E. coli (Figure 2). At pH 6, experimental results were similar but the difference in killing was more pronounced at one minute (Figure 3).

With 0.25 mg/L free chlorine initially, after one minute of contact, killing of C. jejuni was greater than for E. coli under several test conditions (Table 3). With 0.5 mg free chlorine initially, killing of C. jejuni was equal to or greater than the killing of E. coli under the same conditions shown in Table 3. To determine whether the PEN 1 strain was representative, we also studied two other C. jejuni strains (Table 4). These strains were more susceptible than the PEN 1 and the E. coli strains.

DISCUSSION

Biocidal killing by chemical disinfectants is a kinetic process (8). When free chlorine-containing fluids are placed in contact with susceptible microorganisms two phenomena are occurring simultaneously: killing of the organisms and binding of the free chlorine to the organic material. The biocidal reaction will continue to occur until all the cells are killed or until all the free chlorine is bound. Because of this kinetic relationship we have chosen to conduct our disinfection experiments with monitoring of both viable organisms and free residual as the reaction progresses. Rather than conclude that killing has occurred when a certain free chlorine concentration is introduced into the suspension containing the test bacteria, we examined the interaction throughout the reaction. Chlorine-binding to test bacteria appeared quickly in the disinfection period; the presence of an exogenous source of demand in this assay system could not be ruled out.

Against inocula of 103 to 106 C. jejuni cfu/ml, initial free chlorine concentrations of 0.25 mg/L were completely effective for inactivation within 5 minutes. Because of the rapidity of inactivation at this level we were unable to discern an effect of pH or temperature on the reaction. Studies of lower free chlorine concentrations may permit analysis of the pH- and temperature-dependency of this reaction.

We found that under the experimental conditions studied, the C. jejuni PEN 1 strain was equal to or more susceptible to the bactericidal effects of chlorine as the E. coli test strain. Limited studies with two other C. jejuni strains showed a similar phenomenon. These results indicate that chlorination procedures that effectively inactivate E. coli from water will also be effective for inactivating C. jejuni. Since the reservoir for C. jejuni is feces, in fecally-contaminated water the absence of E. coli after chlorine disinfection should also suggest absence of C. jejuni.

ACKNOWLEDGEMENTS

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REFERENCES

1. BLASER, M.J. ET AL. Campylobacter enteritis in the United States. A multicenter study. Ann. Intern. Med., 98:360 (March 1980).

2. BLASER, M.J.; TAYLOR, D.N.; & FELDMAN, R.A. Epidemiology of Campylobacter jejuni infections. Epidemiol. Rev., 5:157 (Sept 1983).

3. TAYLOR, D.N. ET AL. Campylobacter enteritis from untreated water in the Rocky Mountains. Ann. Intern. Med., 99:38 (July 1983).

4. WANG, W-L.L. ET AL. Studies of the effects of disinfectants on Campylobacter jejuni. Appl. Environ. Microbiol., 45:1202 (April 1983).

5. PENNER, J.L., & HENNESSEY, J.N. Serotyping Campylobacter fetus subsp. jejuni on the basis of somatic (O) antigens. J. Clin. Microbiol., 12:732 (Dec 1980).

6. PETERSON, D.A. ET AL. Effect of chlorine treatment on infectivity of hepatitis A virus. Appl. Environ. Microbiol.; 45:223 (Jan 1983).

7. AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for the examination of water and wastewater, 15th edition. APHA, Washington, D.C., 1980, Section 408.

8. HOFF, J.C. & GELDREICH, E.E. Comparison of the biocidal efficiency of alternative disinfectants. Journl A.W.W.A. 73:40 (Jan 1981).

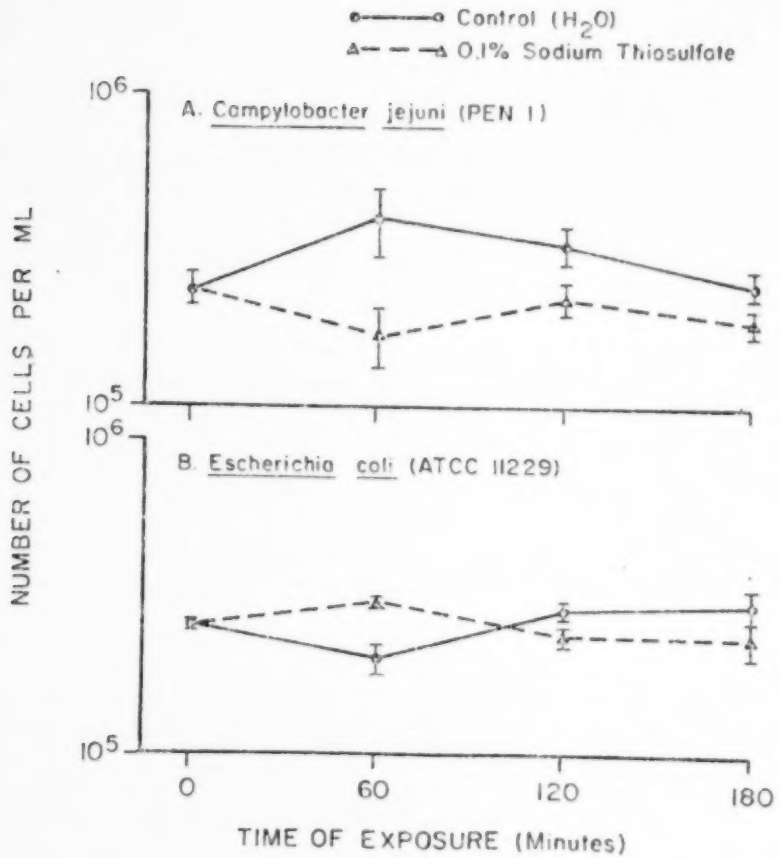

Figure 1: Survival of Campylobacter jejuni and Escherichia coli in 0.1% sodium thiosulfate at 5oC and pH 7.0. There is no significant killing of the test strains by the neutralizer of halogens. Points shown are means ± standard error of triplicate experiments.

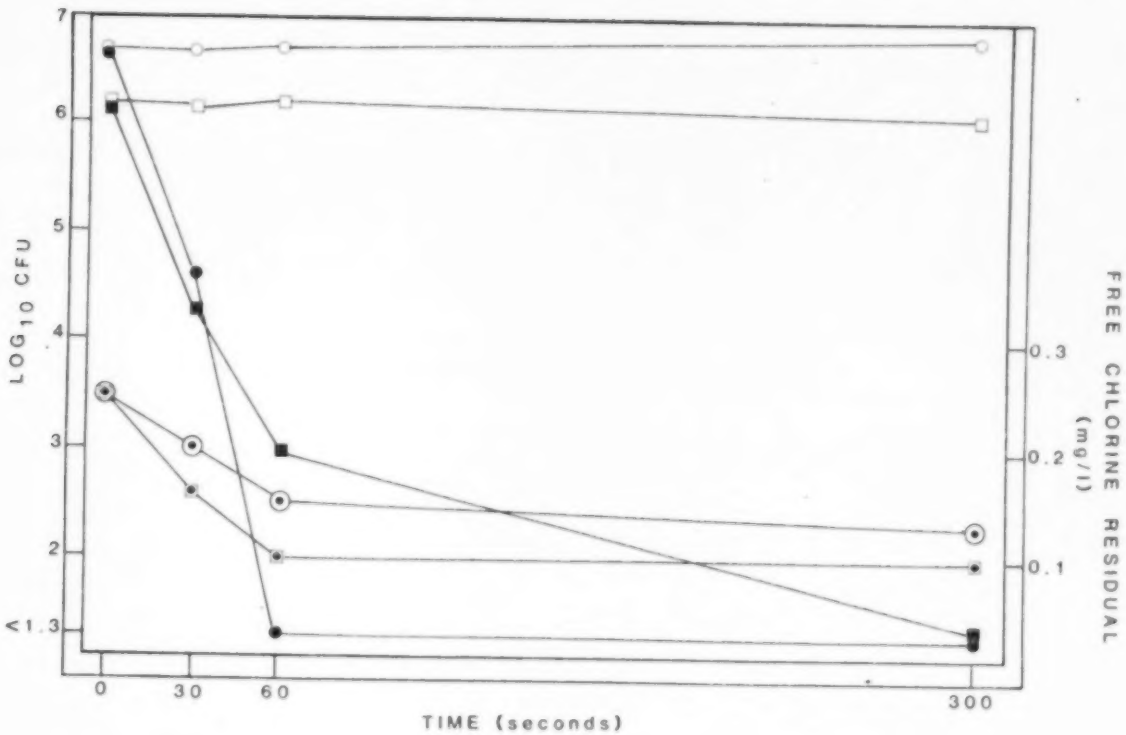

Figure 2: Effect of free chlorine on Campylobacter jejuni PEN 1 and Escherichia coli ATCC 11229 at pH 8.0 and 5oC. Symbols are: E. coli incubated with buffer (□); C. jejuni incubated with buffer (O); E. coli incubated with chlorine (■); C. jejuni incubated with chlorine (●); free residual chlorine in E. coli suspension (□); free residual chlorine in C. jejuni suspension (O). When no orgtanisms were recovered, data point is represented as <1.3 log10 cfu.

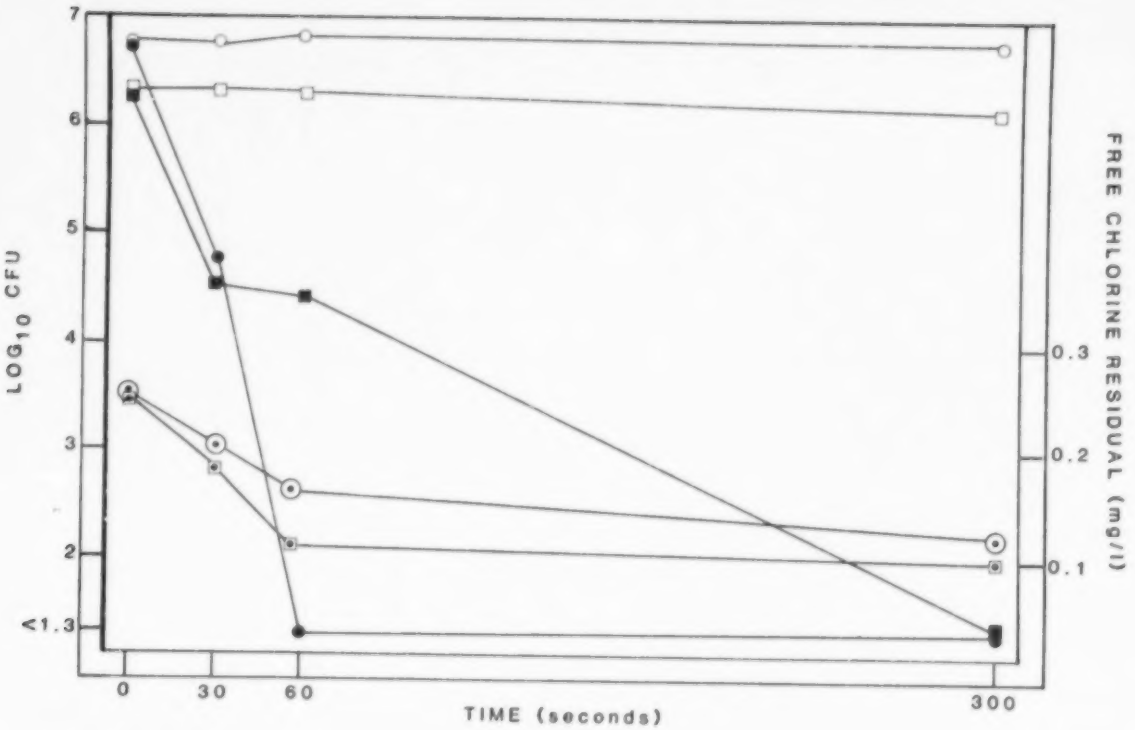

Figure 3: Effect of free chlorine on Campylobacter jejuni PEN 1 and Escherichia coli ATCC 11229 at pH 6.0 and 5°C. Symbols are: E. coli incubated with buffer (□); C. jejuni incubated with buffer (○); E. coli incubated with chlorine (■); C. jejuni incubated with chlorine (●); free residual chlorine in E. coli suspension (▣); free residual chlorine in C. jejuni suspension (⊙). When no organisms were recovered, data point is represented as <1.3 $\log_{10}$ cfu.

Table 1

CHLORINE DEMAND BY 106 cfu/ml *E. COLI* ATCC 11229 AT 5oC

	Free Chlorine (mg/L) at PH		
Time (min)	6	7	8
0	0.50	0.50	0.50
5	0.38	0.30	0.30
15	0.24	0.26	0.24
30	0.15	0.26	0.19

Table 2

CHLORINE DEMAND BY 10^6 cfu/ml *C. JEJUNI* PEN 1 AT 5°C

Time (min)	Free Chlorine (mg/L) at pH 6	7	8
0	0.55	0.55	0.55
5	0.25	0.25	0.22
15	0.10	0.15	0.12
30	0.15	0.12	0.12

Table 3

COMPARISON OF INACTIVATION OF E. COLI ATCC 11229 AND C. JEJUNI PEN 1 AT ONE MINUTE BY 0.25 MG/L FREE CHLORINE

pH	Temp (°C)	Log_{10} killing	
		E. coli	*C. jejuni*
6	5	1.8	>4.1
6	25	NKa	>3.7
8	5	1.9	>4.1
8	25	1.2	>3.6

aNK, no killing detected

Table 4

COMPARISON OF INACTIVATION OF E. COLI AND C. JEJUNI AT ONE MINUTE AT 5oC BY 0.5 MG/L

Strain		Log_{10} kill at pH 6	pH 8
E. coli ATCC 11229		2.3	2.2
C. jejuni	PEN 1	>4.3	>1.5
	PEN 2	1.9	>0.9
	PEN 3	>3.7	>3.0